Monitoring Cell Migration

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MR cellular imaging is a relatively new and emerging technique aimed at guiding development of effective cell therapies, including progenitor cells, stem cells, and immunotherapeutic cells. It provides the opportunity to non-invasively map grafted cells in living animals and humans, to observe their migration and, more importantly, to link functional measures with the presence of the cells in target organ. This technique also addresses the safety issue by monitoring potential uncontrolled proliferation or dispersion to unwanted areas. At the present time, MR cellular imaging now allows whole-body scanning capability at a resolution approaching the size of individual transplanted cells (1, 2), while allowing repeated analysis at multiple time points.

Numerous techniques have been developed to magnetically label cells prior to grafting, most of them utilizing dextran-coated superparamagnetic iron oxide nanoparticles (SPIOs). SPIOs can be used to label cells from multiple species, simply by 24-48 hours of incubation in conjunction with transfection agents (3) or, as has been recently has been shown, by instant labeling using magnetoelectroporation (4), allowing for non-invasive detection of transplants using MRI. Most recent studies have employed Feridex as SPIO, as it is a commercially-available and FDA-approved source of dextran-coated SPIO. Gd-chelates have also been used (5-7), albeit in much less extent, owing to the reduced sensitivity of the paramagnetic label once internalized in cells, forcing the use of T2(*)-weighted hypointensity imaging at higher field strengths. A large number of gadolinium molecules are needed in each cell to allow a robust *in vivo* visualization, although some strategies, such as metallofullerines (8), have been described to increase relaxivity and therefore could reduce the number of paramagnetic molecules needed for cellular imaging. Daldrup-Link (9) demonstrated that twice as many cells labeled with gadopentate dimeguline liposomes were needed for detection by MRI compared to ferumoxides, P7728 liposomes or polysaccharide nanoparticles.

Most interest to date in the use of pre-labeled cells for cellular MR imaging has been in the use of stem cells. The *ex vivo* MR analysis of the global or local distribution of transplanted stem cells is now a routinely used method that complements conventional histological techniques. This method allows for 3D whole body or organ imaging and presentation of data in any desired anatomical plane—features that are impossible or extremely difficult to achieve using conventional microscopy. Post-mortem (ex vivo) imaging is not subject to the MR technique limitations associated with living organism imaging (limited acquisition time or motion artifacts); thus, the spatial resolution, signal-to-noise ratio, and sensitivity are much better. The MRI cell tracking technique, however, offers far more valuable data when used in a living organism, allowing for dynamic evaluation of cell migration. From a clinical perspective, it also offers a non-invasive means of monitoring cell-based therapy that enables determining the cell

fate without the need for tissue biopsy. Brain and cardiac disorders have received so far the most attention for the potential application of MRI stem cell tracking techniques (10).

Monitoring immune cell trafficking is the second major application of MR imaging of cell migration. It is likely to have a significant future impact on our understanding of the in vivo immune response. Anderson et al. have demonstrated targeted trafficking of myelin—sensitized T cells into the spinal cord of animals with experimental allergic encephalomyelitis (11). Antigen-specific cytotoxic T cells have been found to specifically home to OVA+ (12) and HER2/neu tumors (13). T cells from animals that rejected OVA^+ tumors have been purified, SPIO-labeled, and re-injected in OVA^+ tumor-bearing animals (14). In this case, specific tumor acumulation could also be observed. MR monitoring of cytotoxic T cell migration into the diabetic pancreas has been pursued in order to gain insight into the mechanisms underlying autoimmune diabetes (15, 16). SPIO-labeled cells behave clearly different from naked SPIO particles when injected intravenously; Endorem-labeled hematopoietic bone marrow cells were found to rapidly home to bone marrow following intravenous injection whereas just the Endorem particles by themselves do not (17).

While MR imaging is rapidly gaining ground for cellular imaging, other techniques also possess the potential for cellular imaging in the living organism. Especially studies on hematopoietic stem cell transplantation have used fluorescent dyes, such as PKH26, to pre-label cells *in vitro* to allow *in vivo* detection of transplanted/infused cells by means of optical imaging (18-20). However, this approach does not allow deep tissue penetration and therefore is limited to easily accessible targets. Alternatively, invasive procedures, such as a craniectomy, are required to allow access of the imaging probe to the anatomical structure of interest (18). Exposure to light to visualize the fluorescent dyes can also cause phototoxicity (21) killing the labeled cells complicating the serial assessment of these cells by optical imaging methods. The attractiveness of this approach lies in the commercial availability of fluorescent dyes for cellular tacking that are widely used on a variety of cell types and provides a powerful approach to study, for instance, cellular differentiation by combining the fluorescent marker for the identification of transplanted cells with immunohistochemical methods.

A promising new development in cellular imaging consists of the possibility to detect bioluminescence from deep tissue structures (22). Typically, a gene encoding for luciferase, is engineered into cells (23). The substrate luciferin (supplied through i.v. injection) is oxidized by luciferase in the presence of ATP and oxygen, producing a photon in the course of this bioluminescent reaction that can be detected by very sensitive cameras, thus allowing repeated in vivo cellular imaging (24). A drawback of bioluminescent imaging is that the scattering of light by tissue limits the spatial resolution of this technique considerably compared to MRI. In addition, clinical or whole body large animal imaging is, at present, not feasible, and pigmented strains are less suitable for bioluminescent imaging, raising ethical issues would the technology allow imaging in humans. Still, bioluminescent imaging has allowed Kim and colleagues (25) to track the *in vivo* migration of neural stem cells from the contralateral hemisphere to the site of lesion in mice with middle cerebral artery occlusion whilst allowing a corroboration of these results by histology. Migration of neural stem cells into gliomas across hemispheres has also been visualized (26), and the trafficking of immune cells has been monitored as well (27, 28). The potential to track a particular type of cell, such as a neural stem cell or immune cell, by bioluminescence, whilst using the same techniques to detect light emitted at a different wavelength from distinct reporter genes to study gene expression in these cells clearly will provide interesting novel avenues to study the basic biology of transplanted cells by combining

molecular (gene expression) and cellular imaging (location of cells).

Various other techniques, such as positron emission tomography (PET) and single photon emission tomography (SPECT), also have the potential to visualize cells and cell migration *in vivo*. The current spatial resolution of both PET and SPECT is at least 10 times lower than that observed with MRI, but it is the higher specificity of radioligands that conveys the attractiveness of these techniques to study transplanted cells *in vivo* (29). For instance, Jacobs et al (30) have shown that engineering of reporter genes into cells in vitro allows the identification of these cells by means of detection of the transgene expression *in vivo*. Hence, the reporter molecules could serve as a surrogate marker of the cells to be imaged and no prior loading of the cells with a contrast agent would be required as radioligands are small enough to cross the intact blood-brain barrier. Differential expression of proteins/receptors, such as the transferrin receptor, on particular cells can also serve as a marker to identify these transplanted cells *in vivo* (31, 32). Although both PET and SPECT provide excellent approaches to study specific molecular targets and determine cellular differentiation or metabolic consumption, the repeated assessment of animals with radioligands raises issues regarding the safety of this approach for longitudinal studies and its translation into a clinical setting. The pre-labeling of cells *in vitro* with radioligands prior to in vivo imaging (as exemplified by MR cellular imaging) is also less likely to be apposite for the longitudinal tracking of cells due to the short half-life of radioligands. Nonetheless, Chin et al. (33) have demonstrated that it is possible to visualize mesenchymal stem cells by SPECT over a period of 14 days after pre-incubation of cells with ^{111}In oxine. Kraitchman et al. (34) clearly demonstrated the much higher sensitivity of detecting mesenchymal stem cells with ^{111}In oxine as compared to labeling with Feridex; estimated numbers of between 50,000-100,000 cells could be detected as hot spots on SPECT/CT images but not on the MR images (using clinically applicable imaging parameters). Another cell tracking approach is the use of specific reporter markers in transplanted cells that are detectable by repeated administration of a radioligand thus providing an attractive approach to monitor immune (35, 36) or stem cell (37) graft survival and function over extended time periods.

However, the need for on-site synthesis of radioligands limits the wider applicability and practicality of PET and SPECT as cellular imaging techniques, whereas limits on tissue penetration and need for genetic engineering will complicate translation of optical imaging and bioluminescence into clinical applications. With MR imaging, it is impossible to differentiate live from dead cells, and label dilution by cell division is a significant limitation. But the wider availability of MRI scanners in both clinical and pre-clinical settings strengthens the applicability of cellular MR imaging as a translational technique that can be easily implemented in many centers. Given the high-resolution of MR imaging at near cellular levels, however, it is the unique complementary information that can be derived from the different imaging approaches that highlight their importance in cellular imaging. This is well illustrated by the first clinical trial that has used magnetically labeled cells (38): MR imaging was superior to ^{111}In-oxine scintigraphy in detecting the number of lymph nodes that Endorem-labeled dendritic cells migrated to following intranodal injection in a single regional node, and was able to accurately asess whether the nodes were hit or missed following US-guided injections. At the other hand, only scintigraphy allowed accurate quantification of migrated cell numbers, which is important for predicting sufficient T cell activation.

For cellular MR imaging, a few new approaches were introduced last year that may offer opportunities for improved monitoring of cell migration. Gilad et al. in our group have developed an MR reporter gene, that is unique in the sense that it is an artificial reporter gene providing

endogenous contrast without the need for administration of contrast agents (39). Using chemical exchange saturation transfer (CEST) imaging, images can be created using specific off-radiation pulses that null the signal of amide protons in the reporter gene. By subtracting and correcting the anatomical images without the specific pulse, "hot spot" images can be created that have now allowed accurate detection of cell distribution in vivo. Succesful development of MR reporter genes would have the same advantages that bioluminescent and PET imaging have when using reporter genes: 1) no decrease of the sensitivity of detection following cell division (caused by dilution of label); 2) the ability to discriminate between live and dead cells (as only live cells continue to produce the reporter); and 3) the potential to place reporters under specific promotors thus allowing the detection of cells only under certain activation or differentiation conditions (for instance, the visualization of mesenchymal stem cells only when differentiated into cardiomyocytes). Cohen et al. (40) Genove et al. (41) have used a different approach that still relies on metal-based relaxation changes, namely the use of a ferritin reporter gene, synthesizing its own iron oxyhydroxide core, that could possibly also be used for cell tracking.

Finally, ^{19}F-based "hot spot" MR imaging has emerged from the background. Perhaps largely unrecognized, fluorine-19 was one of the earliest applied MR contrast agents (42). Ahrens et al. (43) have used large particles containing many ^{19}F atoms to label dendritic cells, and demonstrated that organ trapping in the liver, spleen and lungs could be readily visualized. As compared to ^{1}H imaging and using metal-based contrast agents, ^{19}F-based imaging offers several advantages but may also have some pitfalls, which have recently been discussed in further detail (44). Thus, there are many ways to monitor cell migration. It is too early to tell which of all the described techniques will become the future technique of choice, but it is clear that there is an array of possibilities with the optimal choice depending on the specific cell tracking application.

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